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TESTING ALTERNATIVE HYPOTHESES OF *NEOTRIGONIA* (BIVALVIA: TRIGONIOIDA) PHYLOGENETIC RELATIONSHIPS USING CYTOCHROME C OXIDASE SUBUNIT I DNA SEQUENCES

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ABSTRACT

Uncertainties regarding the phylogenetic history within the Bivalvia have impeded attempts to understand evolution within the group. Estimating the evolutionary relationships surrounding the Trigonioida has been especially problematic and has led to disparate hypotheses regarding (1) the origin and subsequent diversification of unionoid bivalves and (2) autobranch gill character state transitions. In order to test alternative hypotheses of trigonioid phylogeny, 613 base pairs of DNA sequence of the cytochrome *c* oxidase subunit I gene were analyzed from 14 species of bivalves (ingroup) and three species of non-bivalve mollusks (outgroup). All phylogenetic analyses, using either nucleotide or inferred amino acid sequences, produced trees that robustly placed *Neotrigonia* (Trigonioida) as the sister taxon to a monophyletic Unionoida. Furthermore, the Autobranchia, Veneroida, and Mytiloida were supported as monophyletic groups in these analyses, whereas the Bivalvia was not. These phylogenetic relationships suggest that (1) there was a single invasion of freshwater by a unionoid bivalve ancestor, (2) trigonioid rather than veneroid bivalves gave rise to the Unionoida, (3) either the eulamellibranchous or filibranchous gill condition has evolved multiple times within the Autobranchia, and (4) the molluscan bivalved body plan may have evolved more frequently than traditional phylogenetic hypotheses suggest.

Key words: Bivalvia, Trigonioida, Unionoida, cytochrome *c* oxidase I, mtDNA, phylogenetics, convergence.

INTRODUCTION

The higher-level phylogenetic relationships within the Bivalvia are poorly understood at present (e.g., Allen, 1985). This statement is corroborated by the many disparate hypotheses of evolutionary relationships that have been proposed for the higher taxa within the Bivalvia (e.g., Purchon, 1990; Waller, 1990; Starobogatov, 1992; Cope, 1996; B. Morton, 1996; Salvini-Plawen & Steiner, 1996). This plethora of phylogenetic hypotheses likely stems, in part, from arbitrary choices to exclude certain types of characters and a general lack of explicit and rigorous phylogenetic analyses. Although major anatomical character suites distinguish two of the nominal subclasses within the Bivalvia, that is, Protobranchia and Autobranchia, the ordinal-level relationships within each subclass are not strongly supported by multiple shared-derived morphological features (e.g., Waller, 1990).

Two contrasting phylogenies of the higher-level relationships within the Bivalvia have recently been proposed by Salvini-Plawen & Steiner (1996; Fig. 1A) and Waller (1990; Fig. 1B). The former study is an exemplar in that explicit data matrices and phylogenetic methodologies were presented. A major point of disagreement between the two phylogenies presented in Figure 1 is the placement of the Trigonioida, a relatively ancient bivalve taxon that, although currently depauperate, was taxonomically diverse during the Mesozoic (Cox et al., 1969; Allen, 1985). The Salvini-Plawen & Steiner hypothesis (Fig. 1A) indicates that the trigonioids are most closely related to pteriomorph bivalves (represented herein by mytiloids), with the Veneroida closely related to the Unionoida. Their proposed sister taxon relationship for the trigonioid and pteriomorph bivalves was supported by the shared presence of (1) byssate larvae and adults and (2) abdominal sense organs in these taxa (Salvini-Plawen & Steiner, 1996). Alternatively, the hypothesis of Waller (Fig. 1B) indicates that the Trigonioida is the sister taxon to the Unionoida. However, no evidential sup-

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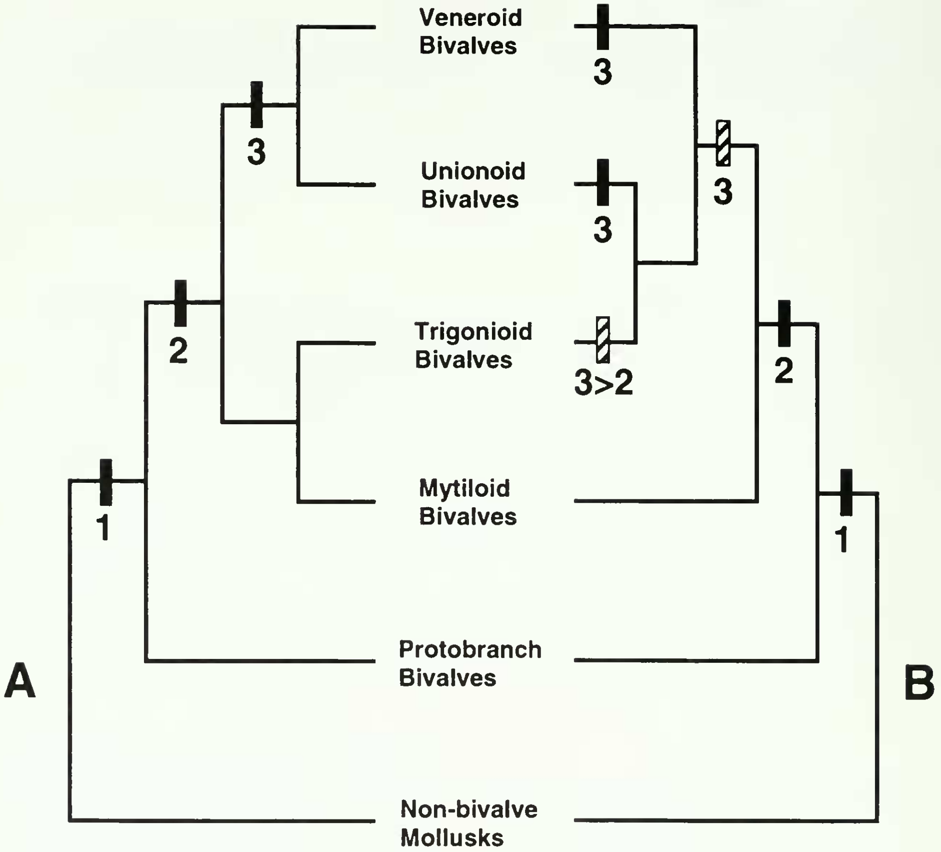

FIG. 1. Bivalve relationships, based on morphological characteristics, according to Salvini-Plawen & Steiner (1996; A) and Waller (1990; B). Both hypotheses indicate a single origin for the Bivalvia (1) and filibranchous lamellibranch gills (2) but differ in their implications for eulamellibranch gill evolution. Hypothesis A indicates a single origin for eulamellibranch gills (3) while B indicates either two origins (solid bars) or a single origin followed by a reversal to the filibranch condition in trigonioids (hatched bars).

port for this relationship was provided by Waller (1990).

The evolutionary affinities of the Trigonioida have been a contentious subject for over a hundred years (e.g., Steinmann, 1888; Neumayr, 1889; Cox, 1960; B. Morton, 1987; Healy, 1989, 1996) and are central to an understanding of bivalve mollusk character evolution, since representatives of the single extant genus (*Neotrigonia*) have a mixture of seemingly primitive (e.g., filibranchous gills [possessing ciliary-linked gill filaments], lack of posterior mantle fusion, nacreous shells; Cox, 1960; Allen, 1985; B. Morton, 1987) and derived features (e.g., multi-vesicular sperm acrosome; Healy, 1989). This mosaic of seemingly primitive and derived character states has contributed to the erection of multiple, disparate hypotheses of trigonioid evolutionary affinities. According to the hypothesis of Salvini-Plawen & Steiner (1996; Fig. 1A), the placement of the trigonioids is consistent with a single origin of eulamellibranch gills (tissue-linked gill filaments) from filibranch gills. In contrast, the phylogenetic placement of trigonioids in the hypothesis of Waller (1990; Fig. 1B) suggests that there were either (1) two origins of eulamellibranch gills or (2) a single origin of eulamellibranch gills followed by a reversal to filibranch gills in the trigonioids.

The phylogenetic uncertainty within the Bivalvia impedes the rigorous testing of evolutionary hypotheses. Therefore, the establishment of a robust phylogenetic hypothesis for higher taxa within the Bivalvia will enable critical evaluations of (1) trigonioid evolutionary relationships and (2) hypotheses of autobranch bivalve gill character state transitions. Molecular systematic analyses have been useful in situations where morphological analyses were inconclusive (e.g., Avise, 1994). Therefore, mitochondrial DNA (mtDNA) sequences, obtained from the cytochrome *c* oxidase subunit I (COI) gene, were used to construct a phylogenetic hypothesis for the major bivalve lineages represented in Figure 1. COI was chosen for this analysis because of its slow rate of evolution relative to other mitochondrial protein coding genes, relative ease of unambiguous sequence alignment (e.g., Brown, 1985; Simon et al., 1994; Russo et al., 1996), and demonstrated appropriateness for this particular analysis.

MATERIALS AND METHODS

Organisms

The molluscan taxa used in this study, with GenBank accession numbers and primary literature citation (where applicable), are as follows: (1) ingroup, Class Bivalvia, Subclass Protobranchia, Order Solemyoida, *Solemya velum* (U56852), Order Nuculoida, *Nucula tenuis* (U56851), Subclass Autobranchia, Order Unionoida, *Mutela rostrata* (U56849), *Amblema plicata* (U56841), *Anodonta cygnea* (U56842), *Margaritifera margaritifera* (U56847), Order Trigonioida, *Neotrigonia margaritacea* (U56850), Order Mytiloida, *Modiolus modiolus* (U56848), *Geukensia demissa* (U56844), Order Veneroida (the five veneroid COI sequences are from Baldwin et al. [1996]), *Corbicula fluminea* (U47647), *Rangia cuneata* (U47652), *Mercenaria mercenaria* (U47648), *Mytilopsis leucophaeata* (U47649), *Dreissena polymorpha* (U47653), (2) outgroup, Class Scaphopoda *Dentalium* sp. (U56843), Class Gastropoda *Lepetodrilus elevatus* (U56846), Class Polyplacophora *Katharina* sp. (U56845).

Methods

Total DNA was isolated from somatic (mantle) tissues of nine species of bivalves. Male gonadal tissues were specifically avoided during dissections to prevent comparisons of non-orthologous sequences (due to the actual or potential presence of doubly uniparental inheritance of mtDNA in some bivalve taxa; for example, Skibinski et al., 1994; Zouros et al., 1994; Hoeh et al., 1996). DNA was also isolated from representatives of three additional molluscan classes (i.e., Gastropoda, Polyplacophora, and Scaphopoda) for use in generating outgroup sequences. Subsequently, a 710bp fragment of COI was PCR amplified and cycle sequenced for each of the 12 taxa as described elsewhere (Folmer et al., 1994). Both strands of the COI fragment were sequenced from each of two individuals from each terminal taxon to guard against PCR-based contamination artifacts. The resulting 12 COI sequences, plus the five veneroid bivalve COI sequences from Baldwin et al. (1996), were readily aligned by eye using MacClade 3.05 (Maddison & Maddison, 1992). Sixteen of the 17 OTUs produced sequences 613 bp in length, while that of *Geukensia* was 616 bp. The increased length of the *Geukensia* sequence was due to an autapomorphic, three nucleotide (single codon) insertion event. These three contiguous nucleotides, which are phylogenetically uninformative for the taxa considered herein, were deleted prior to all phylogenetic analyses. No additional hypothesized insertion or deletion events were necessary to obtain the alignment utilized in the subsequent analyses.

The suitability of the COI data set for phylogenetic analyses at the required hierarchical level was evaluated by plotting the substitution pattern of transitions and transversions for each codon position (e.g., Orti & Meyer, 1996). Furthermore, the degree of phylogenetic signal within the COI data set was evaluated using the g_1 statistic of a random tree distribution (from 100,000 random trees; e.g., Hillis, 1991; Hillis & Huelsenbeck, 1992) as implemented in PAUP (Swofford, 1993). Phylogenetic analyses were carried out on the COI nucleotide sequences using the maximum likelihood ([ML], DNAML in PHYLIP 3.5c; Felsenstein, 1993), neighbor-joining ([NJ], MEGA 1.02; Kumar et al., 1993), and maximum parsimony ([MP], PAUP 3.1.1; Swofford, 1993) algorithms. *Katharina*, the only non-conchiferan mollusk taxon, was used to root the resulting topologies. A transition/transversion ratio of 2.0 was utilized in the ML analyses and the gamma distance (alpha = 0.5, using the Tamura-Nei model of nucleotide sequence evolution) was used to generate the pair-wise genetic distances for the NJ analyses. This particular distance takes into consideration among-site substitution rate variation (e.g., Yang, 1996). Furthermore, MP and NJ (again using gamma distances, alpha = 0.5) analyses were conducted on the inferred COI amino acid sequences (using the *Drosophila* mtDNA genetic code). Multiple random terminal taxon addition order runs, combined with global branch rearrangement options, were employed to generate topologies from ML and MP analyses. These options increased the probability of finding the actual best topology under each of the two optimality criteria (e.g., Hendy et al., 1988; Maddison, 1991). The robustness of the resulting topologies was evaluated by bootstrap analyses (1,000 replicates for MP and NJ, 100 replicates for ML).

The best COI-based topology derived from the DNAML analysis was compared with the phylogenetic hypotheses presented in Figure 1 using the Kishino-Hasegawa test (paired z test; Kishino & Hasegawa, 1989) as implemented in DNAML. To this end, the topological constraints option in PAUP was used to generate 82 user trees (all trees ≤ five steps longer than the shortest trees found by PAUP) representing the two tree topologies (for the particular taxa evaluated herein) in Figure 1. Each of these user trees was then compared to the best DNAML tree by the Kishino-Hasegawa algorithm. This test evaluated the significance of any potential incongruence between the morphology- (Figure 1) and COI-based topologies (Swofford et al., 1996). In addition, character optimization, using MacClade (Maddison & Maddison, 1992), was carried out on the COI-based topologies to investigate their implications for morphological character evolution within the Bivalvia.

RESULTS

Scatter plots of the relationship between the number of transitional and transversional substitutions at each of the three codon positions and the proportion of nucleotide differences (all positions) for the COI sequences revealed that only transitional substitutions at the third codon position had reached saturation (Fig. 2). Because saturated categories of substitution can contribute to erroneous estimates of evolutionary history (e.g., Swofford et al., 1996), all first and second position substitutions together with only transversions at the third codon position were included in the COI nucleotide data matrix used for phylogenetic analyses. Of the 613 nucleotide positions in the transformed COI data matrix, 229 were invariant, while 315 (102 from 1st codon positions; 59 from 2nd; 154 from 3rd) were phylogenetically informative using the parsimony criterion. Analysis of the tree length distribution of 100,000 randomly generated trees, using all 17 taxa, suggests that there is a significant amount of hierarchical structure within the transformed COI data set (g_1 = −0.794; with 384 variable sites, $p << 0.01$; Hillis & Huelsenbeck, 1992). This analysis was repeated using only the *Corbicula*, *Dentalium*, *Katharina*, *Lepetodrilus*, *Modiolus*, *Neotrigonia*, and *Solemya* COI sequences (seven taxa) in order to minimize the number of closely related taxa present in the hierarchical structure analysis. Significant structure was still present in this truncated COI data matrix (g_1 = −0.527; with 295 variable sites, $p < 0.01$), which suggests that the hierarchical structure present in the original data matrix was not solely due to the presence of closely related taxa. The findings from the plots of substitution pattern and g_1 statistics are consistent with the hypothesis that significant phylogenetic signal exists in the transformed COI nucleotide data matrix and validate the latter's use in this particular phylogenetic context (e.g., Swofford et al., 1996). Since amino acid substitution rates are lower than the underlying nucleotide substitution rates (e.g., Li & Graur, 1991), it follows that the inferred COI amino acid sequences are not saturated and, therefore, also appropriate for this level of phylogenetic analysis.

The best tree produced by ML analysis of the transformed COI nucleotide matrix is presented in Figure 3 along with bootstrap percentages for the NJ (above branches, 1,000 replicates), MP (below branches, 1,000 replicates), and ML (in parentheses, 100 replicates) analyses. Only bootstrap percentages greater than 50% are shown. This topology is largely congruent with the best trees produced by MP (three equally parsimonious trees) and NJ analyses (trees not shown). However, the topological relationships of the non-autobranch taxa on the best MP trees were identical to those portrayed in the trees derived from analyses of the inferred COI amino acid sequences (see below).

The Kishino-Hasegawa test results (Table 1) indicate that the morphology-based topology represented in Figure 1A was significantly worse ($p < 0.05$) than the best topology from ML analyses of the transformed COI data matrix (Figure 3). However, the morphology-based topology represented in Figure 1B was

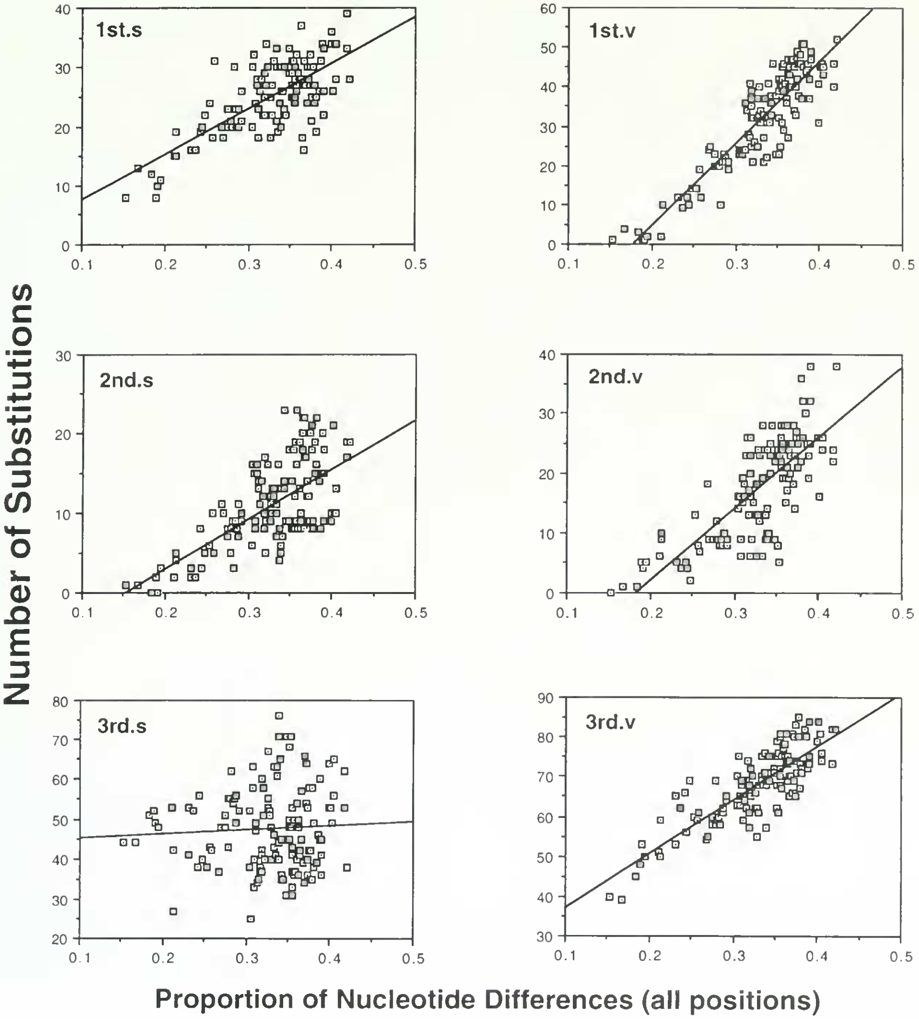

FIG. 2. Substitution pattern for transitions (s) and transversions (v) at each codon position for the COI fragment analyzed herein. The number of transitions and transversions is plotted against the proportion of nucleotide differences over all positions.

not significantly worse ($p > 0.05$) than that derived from ML analysis. These test results confirm significant discordance between the morphology-based topology represented in Figure 1A and the COI-based topology (e.g., Swofford et al., 1996).

Figure 4 represents the best topology generated from NJ analysis of the inferred COI amino acid sequences with bootstrap percentages for NJ (above branches) and MP (below branches) analyses. Only bootstrap percentages greater than 50% are shown. The strict consensus tree (not shown) of the 39 equally parsimonious trees produced by MP analysis of the inferred COI amino acid matrix is less resolved but entirely congruent with the NJ tree.

All analyses of the transformed COI nucleotide and inferred amino acid matrices were consistent with the monophyly of the Autobranchia, Veneroida, Mytiloida, and Unionoida (e.g., Figs. 3, 4). The trees in Figures 3 and 4 are especially noteworthy due to the strong inference that the filibranchous *Neotrigonia* (Trigonioida) is more closely related to eulamellibranchous freshwater mus-

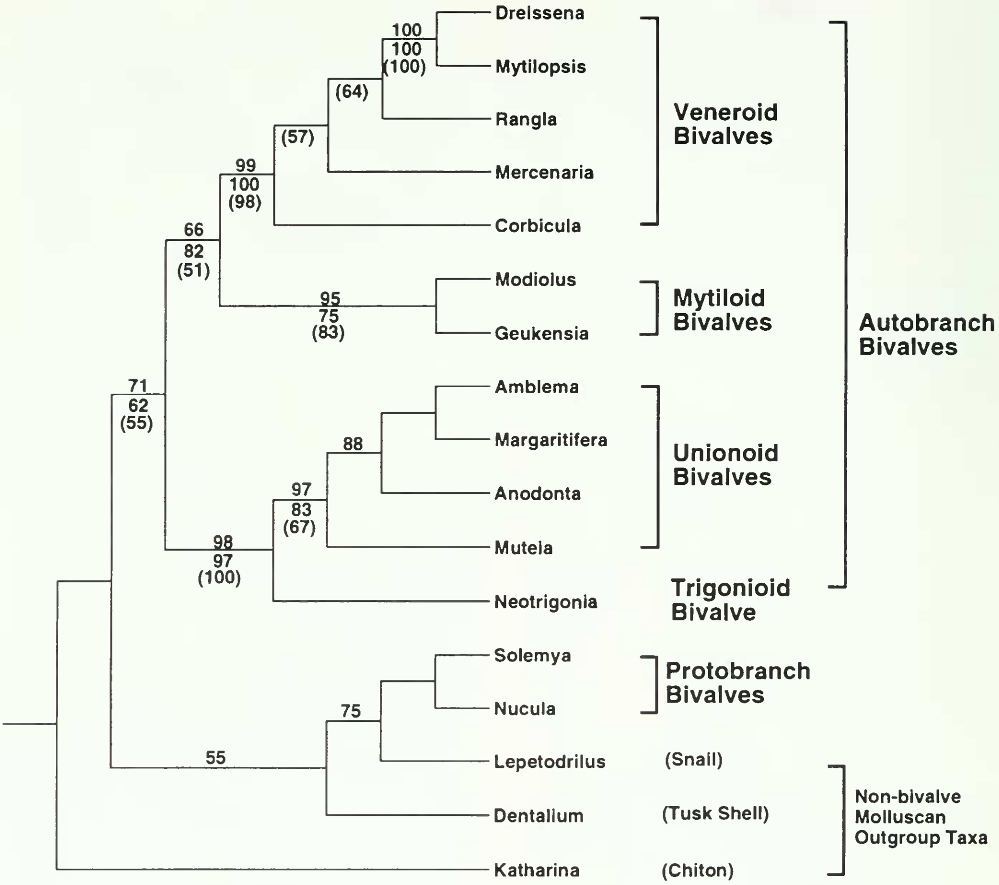

FIG. 3. Best tree topology produced by maximum likelihood analysis of the transformed COI nucleotide matrix. Numerals are bootstrap percentages for NJ (above branches, 1,000 replicates), MP (below branches, 1,000 replicates), and ML (parentheses, 100 replicates) analyses. Only bootstrap values greater than 50% are shown.

TABLE 1. Kishino-Hasegawa Test evaluation of the two phylogenetic hypotheses presented in Figure 1 against the best DNAML tree from the COI nucleotide sequence analysis (Fig. 3). User trees with Z-values (= the difference between log likelihood values of best tree and user tree divided by the standard deviation of the difference) of absolute magnitude 2.0 or greater are considered significantly worse ($p < 0.05$) than the best DNAML tree.

Tree Topology	Log likelihood value (Range of Log likelihood values)	Range of differences in Ln L	Range of Ln L S.D.	Range of Z-values
best DNAML tree	−6579.54852	—	—	—
Figure 1A (34 user trees)	(−6661.59438 to −6634.71091)	−82.04587 to −55.16240	26.3566 to 22.8030	−3.1129 to −2.4191*
Figure 1B (48 user trees)	(−6625.17868 to −6601.80601)	−45.63016 to −22.25749	27.4627 to 18.5040	−1.6615 to −1.2028

*Significantly worse than the best tree topology at the $p < 0.05$ level.

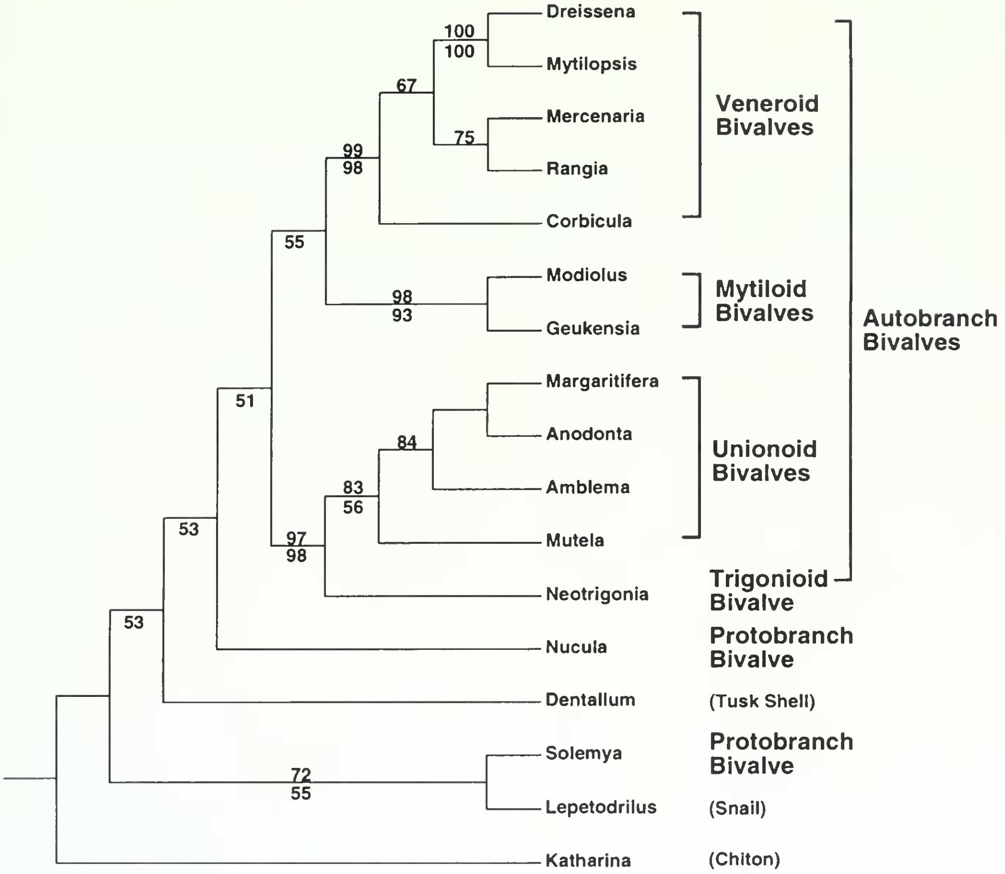

FIG. 4. Best tree topology produced by neighbor-joining analysis of the inferred COI amino acid sequences. Numerals are bootstrap percentages for NJ (above branches, 1,000 replicates) and MP (below branches, 1,000 replicates) analyses. Only bootstrap values greater than 50% are shown.

sels (Unionoida) than to the filibranchous mytiloids (mean level of bootstrap support = 98%). This level of bootstrap support suggests a robust resolution of the evolutionary relationships of *Neotrigonia* among the taxa utilized herein (Hillis & Bull, 1993).

This particular phylogenetic placement of *Neotrigonia* was also manifest in the best trees (not shown) constructed from analyses of the COI nucleotide sequences when (1) the native (untransformed) COI sequences were utilized (ML, MP, and NJ analyses), (2) all codon positions were coded for transversions only (ML, MP, and NJ analyses), and (3) only first and second codon positions were used (ML, MP, and NJ analyses). These results taken together are consonant with the hypothesis that there is a strong phylogenetic signal in the COI sequences supporting the placement of *Neotrigonia* as sister taxon to the Unionoida.

Another noteworthy aspect of all of the above phylogenetic analyses was the absence of support for bivalve mollusk monophyly. In the phylogenetic hypothesis represented in Figure 3, the protobranch bivalves, *Nucula* and *Solemya,* are portrayed as a clade, with the gastropod, *Lepetodrilus,* as the sister taxon to that clade. The phylogenetic hypothesis represented by Figure 4 portrays *Nucula* as the sister taxon of the Autobranchia while *Solemya* is the sister taxon to *Lepetodrilus.* While our analyses were somewhat limited due to the relatively small number of nucleotides and taxa analyzed, the fact that none of the best trees or bootstrap trees generated from these analyses gave support for a monophyletic Bivalvia suggests that the implications of these results be seriously considered.

DISCUSSION

Morton (1987) argued, based partially on the anatomical discontinuities between trigonioids and unionoids, that *Neotrigonia* was a transitional taxon, phylogenetically intermediate between the presumed ancestral protobranch bivalves and the more derived pteriomorph bivalves. This hypothesis is consistent with that of Salvini-Plawen & Steiner (1996; Fig. 1A). However, the results of the COI sequence analyses (Figs. 3, 4) strongly suggest that the extant representative of the Trigonioida, that is, the genus *Neotrigonia,* is the sister taxon to unionoid bivalves, as suggested in the hypothesis of Waller (1990; Fig. 1B). This phylogenetic propinquity is supported by similarities in shell structure (Taylor et al., 1969, 1973; Tevesz & Carter, 1980), gill speculation (Taylor et al., 1969, 1973), sperm morphology (Popham, 1979; Healy, 1989), and gill cilia patterns (Atkins, 1937; Tevesz, 1975). Furthermore, the indicated monophyly of the Unionoida is consistent with the hypothesis of a single invasion of freshwater by the ancestral unionoid. This finding corroborates the hypothesis of a dramatic evolutionary transition in larval morphology, that is, between glochidium and haustorium/lasidium morphology, during unionoid phylogenesis. Evaluating the directionality of this character state transition will require further, broad-scale phylogenetic analyses.

Another interesting result is the placement of *Solemya* (in Figs. 3, 4) and *Nucula* (in Fig. 3), both protobranch bivalves, among the non-bivalve outgroup taxa. This observation is not an artifact of the particular rooting scheme employed in Figures 3 and 4. It is not possible to root either of these topologies such that all of the bivalve taxa represented therein form a monophyletic group. In all of the phylogenetic analyses of the COI sequences, *Solemya* was either (1) the sister taxon to *Lepetodrilus* or (2) in a clade with *Nucula* and *Lepetodrilus.* Therefore, these analyses provide some support for the hypothesis that the currently-recognized molluscan taxon Bivalvia is a polyphyletic assemblage. The non-monophyletic status of the Bivalvia was supported by a recent phylogenetic analysis of 18S rDNA sequences (Adamkewicz et al., 1997: fig. 2). Furthermore, the topology in our Figure 3 suggests that the protobranch bivalve genera *Nucula* and *Solemya* are more closely related to the snail, *Lepetodrilus,* than to the other bivalve taxa in the analysis. Thus, the shared presence of bipectinate gills and hypobranchial glands (J. E. Morton, 1988), esophageal and stomach similarities (Salvini-Plawen, 1988), ultrafiltration site similarities (Andrews, 1988), oxygen transport molecule similarities (Mangum et al., 1987), and flattened pedal areas in both protobranch bivalves and primitive gastropods may be due to shared common ancestry rather than to the retention of ancestral character states.

The phylogenetic relationships of *Neotrigonia, Nucula,* and *Solemya,* as deduced from the COI analyses presented herein (e.g., Figs. 3, 4), suggest that a significant amount of convergent anatomical and conchological evolution has taken place within the Mollusca. There is a great deal of precedent for this statement (e.g., Allen, 1985; Purchon, 1990; Davis, 1994; Salvini-Plawen & Steiner, 1996). An important evolutionary implication that stems from the phylogenetic placement of *Neotrigonia* as sister taxon to a freshwater mussel clade is that it corroborates previous hypotheses of convergence (e.g., Waller, 1990) in the evolution of autobranch bivalve gill structure, i.e., either the filibranchous or the eulamellibranchous gill condition evolved at least twice in the evolutionary history of these bivalve taxa (Fig. 5A). The latter possibility is favored by Waller (1990). The evolution of eulamellibranchous gill organization (which facilitated larval brooding) may have been a necessary antecedent to the successful colonization and subsequent marked evolutionary diversification in freshwater habitats by unionoid bivalves.

The implications of the hypothesized phylogenetic relationships of *Nucula* and *Solemya,* as inferred from analyses of COI sequences, are more profound. It is suggested that the bivalved phenotype has evolved at least three times during the evolution of the Mollusca: (1) in the ancestor of the Juliidae, a relatively derived family within the opisthobranch gastropods (not represented in the analyses herein), (2) in the ancestor of the Autobranchia, and (3) in the ancestor of the Protobranchia (assuming a monophyletic Protobranchia, Fig. 5B). If the genera *Tuarangia* and *Pseudomyona* are found to be bivalved monoplacophorans (Runnegar, 1983) rather than autobranch bivalves (Mackinnon, 1982; Berg-Madsen, 1987), and if the Solemyoida and Nu-

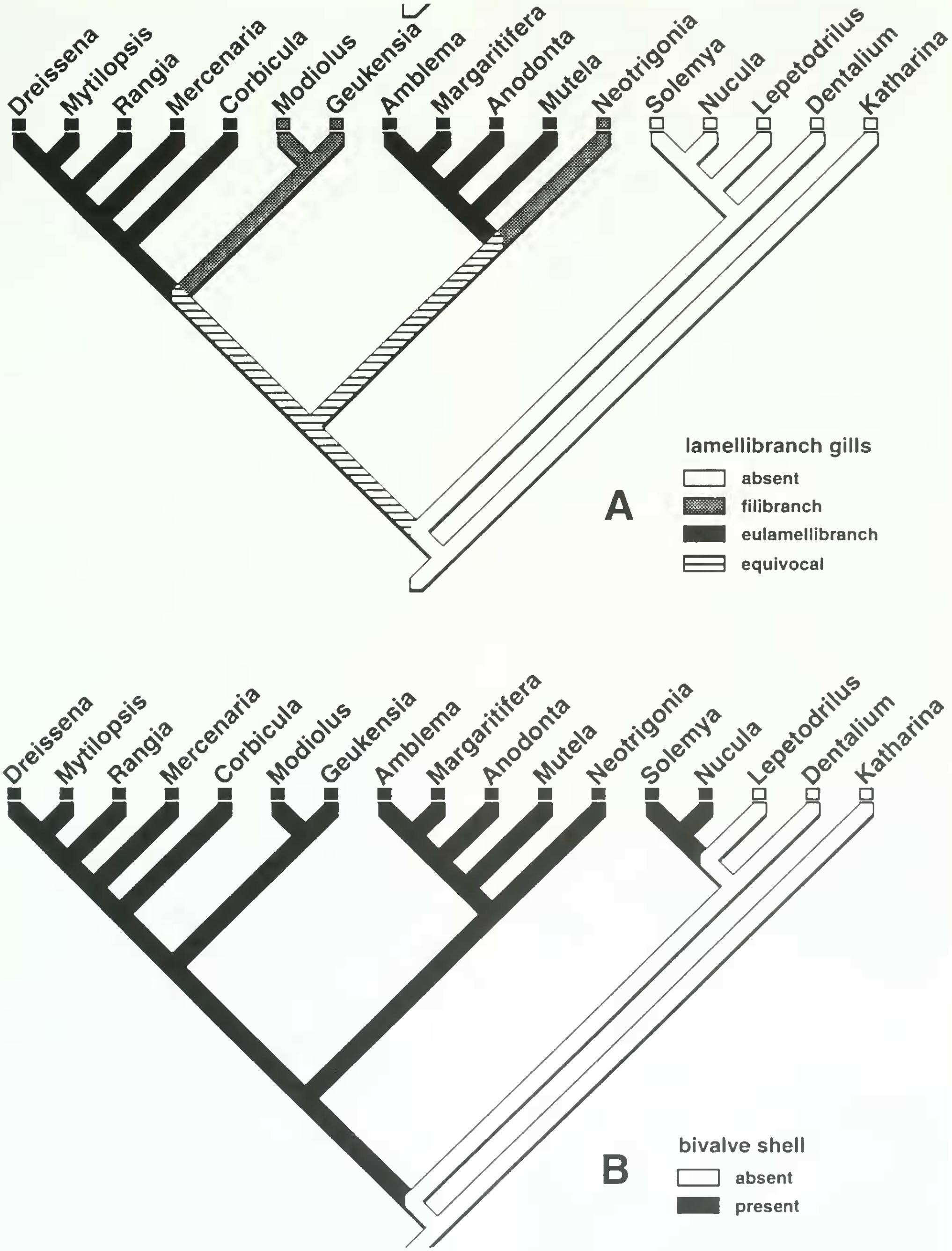

FIG. 5. Morphological character optimization using MacClade on the best topology produced from maximum likelihood analysis of the transformed COI nucleotide matrix using *Katharina* as the outgroup. A. The most parsimonious estimate of autobranch bivalve gill character state transitions for the taxa included in these analyses is that either the filibranchous or eulamellibranchous character state evolved at least twice. B. The most parsimonious estimate of character state transitions for the taxa included in these analyses suggests that the bivalved phenotype has evolved at least twice. Justification for the use of *Katharina* as the outgroup for the molluscan taxa included in this analysis is provided by numerous studies (e.g., Salvini-Plawen, 1980, 1985, 1988, 1990; Wingstrand, 1985; Eernisse, et al., 1992; Salvini-Plawen & Steiner, 1996).

culoida had independent origins (Purchon, 1978; Salvini-Plawen & Steiner, 1996), the bivalved condition would have had to evolved at least five times within the Mollusca. This striking assessment is nonetheless not totally unexpected when evaluated in the context of (1) the great evolutionary mutability of body plan exemplified by the phylum Mollusca (e.g., J. E. Morton & Yonge, 1964; Allen, 1985; Willmer, 1990) and (2) the multiple origins of bivalved external shells in four phyla (Thomas, 1988).

Under the hypothesis of a polyphyletic Bivalvia, the degree of morphological convergence on the bivalved body plan varies considerably within the extant mollusks. In the case of the opisthobranch gastropod genus *Julia,* the shell has converged on the monomyarian (= single adductor muscle) bivalve condition while the soft anatomical characteristics are clearly those of a gastropod (Kay, 1968). However, the degree of convergence in body plans between autobranch and protobranch bivalves is much greater and involves both shell and anatomical character states. The phylogenetic hypothesis displayed in Figure 3 suggests that in the distinct evolutionary histories of the autobranch and protobranch mollusks, in addition to the convergent bivalved shell, there were convergent (1) losses of head and radula, (2)origins of labial palps, (3) origins of the dimyarian adductor muscle condition, and (4) origins of fibrous ligament. Thus, the evolutionary mutability of body plans within the Mollusca may be greater than that suggested by the traditional classification schemes. Phylogenetic evaluations of additional molecular and morphological data sets are needed to test the hypothesized polyphyly of the Bivalvia and further decipher patterns of molluscan body plan evolution.

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LITERATURE CITED

ADAMKEWICZ, S. L., M. G. HARASEWYCH, J. BLAKE, D. SAUDEK & C. J. BULT, 1997, A molecular phylogeny of the bivalve mollusks. *Molecular Biology and Evolution,* 14: 619–629.

ALLEN, J. A., 1985, The Recent Bivalvia: their form and evolution. Pp. 337–403, in E. R. TRUEMAN & M. R. CLARKE, eds., *The Mollusca,* Vol. 10, Evolution. Academic Press, Inc., Orlando, Florida, U.S.A.

ANDREWS, E. B., 1988, Excretory systems of molluscs. Pp. 381–448, in E. R. TRUEMAN & M. R. CLARKE, eds., *The Mollusca,* Vol. 11, *Form and Function.* Academic Press, Inc., San Diego, California, U.S.A.

ATKINS, D., 1937, On the ciliary mechanisms and interrelationships of lamellibranchs. Part II: sorting devices on the gills. *Quarterly Journal of Microscopical Science,* 79: 339–373.

AVISE, J. C., 1994, *Molecular markers, natural history and evolution.* Chapman & Hall, New York, New York, U.S.A. 511 pp.

BALDWIN, B. S., M. BLACK, O. SANJUR, R. GUSTAFSON, R. A. LUTZ & R. C. VRIJENHOEK, 1996, A diagnostic molecular marker for zebra mussels (*Dreissena polymorpha*) and potentially co-occurring bivalves: mitochondrial COI. *Molecular Marine Biology and Biotechnology,* 5: 9–14.

BERG-MADSEN, V., 1987, *Tuarangia* from Bornholm (Denmark) and similarities in Baltoscandian and Australasian late Middle Cambrian faunas. *Alcheringa,* 11: 245–259.

BROWN, W. M., 1985, The mitochondrial genome of animals. Pp. 95–130, in R. J. MACINTYRE, ed., *Molecular evolutionary genetics.* Plenum, New York, New York, U.S.A.

COPE, J. C. W., 1996, The early evolution of the Bivalvia. Pp. 361–370, in J. TAYLOR, ed., *Origin and evolutionary radiation of the Mollusca.* Oxford University Press, London, England.

COX, L. R., 1960, Thoughts on the classification of the Bivalvia. *Proceedings of the Malacological Society of London,* 34: 60–88.

COX, L. R., C. P. NUTTALL & E. R. TRUEMAN, 1969, General features of the Bivalvia. Pp. 2–129, in R. C. MOORE, ed., *Treatise on invertebrate paleontology,* Part N, Vol. 1. The Geological Society of America, Inc. and The University of Kansas, Lawrence, Kansas, U.S.A.

DAVIS, G. M., 1994, Molecular genetics and taxonomic discrimination. *The Nautilus, Supplement,* 2: 3–23.

EERNISSE, D. J., J. S. ALBERT & F. E. ANDERSON, 1992, Annelida and Arthropoda are not sister taxa: a phylogenetic analysis of spiralian metazoan morphology. *Systematic Biology,* 41: 305–330.

FELSENSTEIN, J., 1993, *PHYLIP: Phylogeny inference package, Version 3.5c.* University of Washington, Seattle, Washington, U.S.A.

FOLMER, O., M. BLACK, W. HOEH, R. LUTZ & R. VRIJENHOEK, 1994, DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* 3: 294–299.

HEALY, J. M., 1989, Spermiogenesis and spermatozoa in the relict bivalve genus *Neotrigonia:* relevance to trigonioid relationships, particularly Unionoidea. *Marine Biology,* 103: 75–85.

HEALY, J. M., 1996, Molluscan sperm ultrastructure: Correlation with taxonomic units within the Gastropoda, Cephalopoda and Bivalvia. Pp. 99–113, in J. TAYLOR, ed., *Origin and evolutionary radiation of the Mollusca.* Oxford University Press, London, England.

HENDY, M. D., M. A. STEEL, D. PENNY & I. M. HENDERSON, 1988, Families of trees and consensus. Pp. 355–362, in H. H. BOCK, ed., *Classification and related methods of data analysis.* Elsevier, Amsterdam, Netherlands.

HILLIS, D. M., 1991, Discriminating between phylogenetic signal and random noise in DNA sequences. Pp. 278–294, in M. M. MIYAMOTO & J. CRACRAFT, eds., *Phylogenetic analysis of DNA sequences.* Oxford University Press, New York, New York, U.S.A.

HILLIS, D. M. & J. P. HUELSENBECK, 1992, Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity,* 83: 189–195.

HILLIS, D. M. & J. J. BULL, 1993, An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology,* 42: 182–192.

HOEH, W. R., D. T. STEWART, B. W. SUTHERLAND & E. ZOUROS, 1996, Multiple origins of gender-associated mitochondrial DNA lineages in bivalves (Mollusca: Bivalvia). *Evolution,* 50(6): 2276–2286.

KAY, E. A., 1968, A review of the bivalved gastropods and a discussion of evolution within the Sacoglossa. Pp. 109–134, in V. FRETTER, ed., *Studies in the structure, physiology and ecology of molluscs. Symposia of the Zoological Society of London,* Vol. 22. Academic Press, Inc., London, England.

KISHINO, H. & M. HASEGAWA, 1989, Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order of the Hominoidea. *Journal of Molecular Evolution,* 29: 170–179.

KUMAR, S., K. TAMURA & M. NEI, 1993, *MEGA: Molecular evolutionary genetics analysis, version 1.02.* The Pennsylvania State University, University Park, Pennsylvania, U.S.A.

LI, W-H. & D. GRAUR, 1991, *Fundamentals of molecular evolution.* Sinauer Associates, Inc., Sunderland, Massachusetts, U.S.A.

MACKINNON, D. I., 1982, *Tuarangia paparua* n. gen. and n. sp., a late Middle Cambrian pelecypod from New Zealand. Journal of Paleontology, 56: 589–598.

MADDISON, D. R., 1991, The discovery and importance of multiple islands of most-parsimonious trees. *Systematic Zoology,* 40: 315–328.

MADDISON, W. P. & D. R. MADDISON, 1992, *MacClade: Analysis of phylogeny and character evolution, version 3.01.* Sinauer Associates, Inc., Sunderland, Massachusetts, U.S.A.

MANGUM, C. P., K. I. MILLER, J. L. SCOTT, K. E. VAN HOLDE & M. P. MORSE, 1987, Bivalve hemocyanin: Structural, functional, and phylogenetic relationships. *Biological Bulletin,* 173: 205–221.

MORTON, B., 1987, The functional morphology of *Neotrigonia margaritacea* (Bivalvia: Trigoniacea), with a discussion of phylogenetic affinities. *Records of the Australian Museum,* 39: 339–354.

MORTON, B., 1996, The evolutionary history of the Bivalvia. Pp. 337–359, in J. TAYLOR, ed., *Origin and evolutionary radiation of the Mollusca.* Oxford University Press, London, England.

MORTON, J. E., 1988, The pallial cavity. Pp. 253–286, in E. R. TRUEMAN & M. R. CLARKE, eds., *The Mollusca,* Vol. 11, *Form and function.* Academic Press, Inc., San Diego, California, U.S.A.

MORTON, J. E. & C. M. YONGE, 1964, Classification and structure of the Mollusca. Pp. 1–58, in K. M. WILBUR & C. M. YONGE, eds., *Physiology of Mollusca,* Vol. 1. Academic Press, New York, New York, U.S.A.

NEUMAYR, M., 1889, Uber die Herkunft der Unioniden. *Sitzungsberichte der Oesterreichischen Akademie der Wissenschaften (Wien),* 98: 5–27.

ORTI, G. & A. MEYER, 1996, Molecular evolution of ependymin and the phylogenetic resolution of early divergences among euteleost fishes. *Molecular Biology and Evolution,* 13(4): 556–573.

POPHAM, J. D., 1979, Comparative spermatozoon morphology and bivalve phylogeny. *Malacological Review,* 12: 1–20.

PURCHON, R. D., 1978, An analytical approach to a classification of the Bivalvia. *Philosophical Transactions of the Royal Society of London,* Series B, 284: 425–436.

PURCHON, R. D., 1990, Stomach structure, classification and evolution of the Bivalvia. Pp. 73–82, in B. MORTON, ed., *The Bivalvia: Proceedings of a memorial symposium in honour of Sir Charles Maurice Yonge, Edinburgh.* Hong Kong University Press, Hong Kong.

RUNNEGAR, B., 1983, Molluscan phylogeny revisited. *Memoirs of the Association of Australasian Palaeontologists,* 1: 121–144.

RUSSO, C. A. M., N. TAKEZAKI & M. NEI, 1996, Efficiencies of different genes and different tree-building methods in recovering a known vertebrate phylogeny. *Molecular Biology and Evolution,* 13: 525–536.

SALVINI-PLAWEN, L. V., 1980, A reconsideration of systematics in the Mollusca (phylogeny and higher classification). *Malacologia,* 19: 249–278.

SALVINI-PLAWEN, L. V., 1985, Early evolution and the primitive groups. Pp. 59–150, in E. R. TRUEMAN & M. R. CLARKE, eds., *The Mollusca,* Vol. 10, *Evolution.* Academic Press, Inc., Orlando, Florida, U.S.A.

SALVINI-PLAWEN, L. V., 1988, The structure and function of molluscan digestive systems. Pp. 301–379, in E. R. TRUEMAN & M. R. CLARKE, eds., *The Mollusca,* Vol. 11, *Form and function.* Academic Press, Inc., San Diego, California, U.S.A.

SALVINI-PLAWEN, L. V., 1990, Origin, phylogeny and classification of the phylum Mollusca. *Iberus,* 9: 1–33.

SALVINI-PLAWEN, L. V. & G. STEINER, 1996, Synapomorphies and plesiomorphies in higher classification of Mollusca. Pp. 29–51, in J. TAYLOR, ed., *Origin and evolutionary radiation of the Mollusca.* Oxford University Press, London, England.

SIMON, C., F. FRATI, A. BECKENBACH, B. CRESPI, H. LIU & P. FLOOK, 1994, Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America,* 87: 651–701.

SKIBINSKI, D. O. F., C. GALLAGHER & C. M. BEYNON, 1994, Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis. Genetics,* 138: 801–809.

STAROBOGATOV, Y. I., 1992, Morphological basis for phylogeny and classification of Bivalvia. *Ruthenica,* 2: 1–25.

STEINMANN, G., 1888, [Invertebrates]. In G. STEINMANN, L. DODERLEIN, *Elemente der Palaontologie.* Leipzig, Germany, 848 pp.

SWOFFORD, D. L., 1993, PAUP: *Phylogenetic analysis using parsimony, version 3.1.1.* Smithsonian Institution, Washington, D. C.

SWOFFORD, D. L., G. J. OLSEN, P. J. WADDELL & D. M. HILLIS, 1996, Phylogenetic inference. Pp. 407–514, in D. M. HILLIS, C. MORITZ & B. K. MABLE, eds., *Molecular systematics.* Sinauer Associates, Inc., Sunderland, Massachusetts, U.S.A.

TAYLOR, J. D., W. J. KENNEDY & A. HALL, 1969, The shell structure and mineralogy of the Bivalvia. Introduction. Nuculacea-Trigonacea. *Bulletin of the British Museum (Natural History), Zoology,* Supplement 3: 1–125.

TAYLOR, J. D., W. J. KENNEDY & A. HALL, 1973, The shell structure and mineralogy of the Bivalvia II. Lucinacea-Clavagellacea. Conclusions. *Bulletin of the British Museum (Natural History), Zoology,* 22: 256–294.

TEVESZ, M. J. S., 1975, Structure and habits of the "living fossil" pelecypod *Neotrigonia. Lethaia,* 8: 321–327.

TEVESZ, M. J. S. & J. G. CARTER, 1980, Environmental relationships of shell form and structure of unionacean bivalves. Pp. 295–322, in D. C. RHOADS & R. A. LUTZ, eds., *Skeletal growth of aquatic organisms: biological records of environmental change.* Plenum Press, New York, New York, U.S.A.

THOMAS, R. D. K., 1988, Evolutionary convergence of bivalved shells: a comparative analysis of constructional constraints on their morphology. *American Zoologist,* 28: 267–276.

WALLER, T. R., 1990, The evolution of ligament systems in the Bivalvia. Pp. 49–71, in B. MORTON, ed., *The Bivalvia: proceedings of a memorial symposium in honour of Sir Charles Maurice Yonge, Edinburgh.* Hong Kong University Press, Hong Kong.

WILLMER, P., 1990, *Invertebrate relationships.* Cambridge University Press, Cambridge, England, 400 pp.

WINGSTRAND, K., 1985, On the anatomy and relationships of recent Monoplacophora. *Galathea Reports,* 16: 7–94.

YANG, Z., 1996, Among-site rate variation and its impact on phylogenetic analyses. *Trends in Ecology and Evolution,* 11(9): 367–372.

ZOUROS, E., A. O. BALL, C. SAAVEDRA & K. R. FREEMAN, 1994, An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus. Proceedings of the National Academy of Science, U. S. A.,* 91: 7463–7467.

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